EXHIBIT A

Method for Comparing Percutaneous Absorption of Steroids

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A simple method for assessing the percutaneous absorption of steroid preparations is suggested.

Using vasoconstriction as an index of absorption, occlusion of the skin surface with Saran Wrap results in a 100-fold difference in absorption over simple topical application.

Garb [1] was the first to use thin plastic films in dermatological therapy, and subsequently other workers have testified to the usefulness of plastic film applied over a variety of topical preparations. Scholtz [2] reported good results in recalcitrant psoriasis using fluocinolone cream under Saran Wrap after preliminary abrasion of the lesions with Brasivol. Sulzberger and Witten [3] used triamcinolone acetonide ointment under plastic films and in obstinate psoriasis achieved results which equalled those obtained with intralesional injection of steroid. The latter authors attributed the enhanced activity of steroid applied under a plastic film to better contact between ointment and skin, more accurate localization of the ointment, and increased percutaneous absorption as a result of epidermal maceration and increased skin temperature.

Our own good therapeutic results with this method of treatment led us to attempt a quantitative comparison of the percutaneous absorption of steroid hormones applied to normal skin with and without occlusion. The clinical observation that treatment with topical steroid and Saran Wrap first produced pallor of the lesion and of the surrounding normal skin suggested that vasoconstriction might be used as an index of the percutaneous absorption of the steroid. We could find no report in the literature of the vasoconstrictor activity of steroids when applied to healthy unbroken skin, although Ashton and Cook [4] observed vasoconstriction in super-

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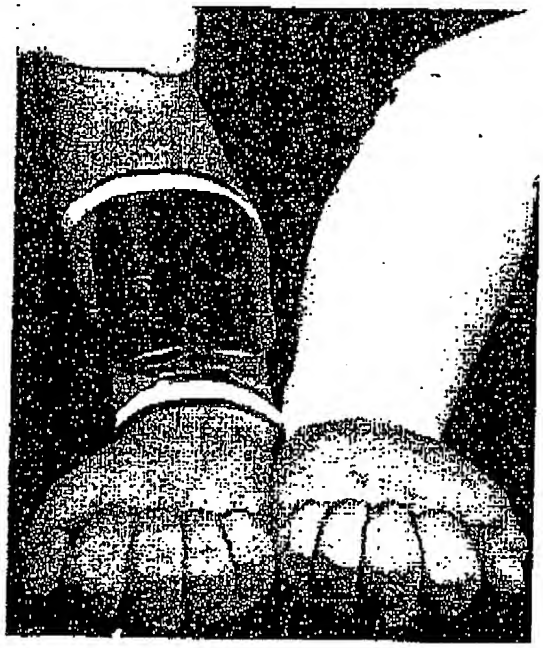

Fig. 1.—Metal guard and Saran Wrap in position.

ficial corneal vascularization treated with subconjunctival steroids, and Hollander et al.[5] reported that intra-articular steroids produced blanching of the engorged synovial membrane in rheumatoid arthritis. It would seem reasonable to postulate that this vasoconstriction is in part responsible for the anti-inflammatory activity of these compounds. Our experiments so far (to be published in full at a later date) indicate that although wide differences exist depending upon the particular steroid and base used, the vasoconstriction produced in normal skin may be useful as an indicator of the percutaneous absorption of steroid preparations.

Method

To assess the effect of Saran Wrap on absorption, 3 steroid preparations were used, dexamethasone alcohol,* triamcinolone acetonide,† and fluocinolone acetonide.‡ Solutions or suspensions of these compounds were prepared in 95% alcohol in dilutions ranging from 1:100 to 1:5,000,000. Using human volunteers, 0.02 ml. of the various dilutions were applied from a dropping pipette on the flexor aspects of both forearms, lightly spread over an area of 1 inch diameter and allowed to dry. To 1 arm was strapped a perforated aluminum guard covering the site of application of the preparations, and the other was wrapped in one layer of Saran Wrap (Dow Chemical Co.) and covered with 2 thicknesses of Tubegauz (Scholl Mfg. Co., N.Y.) (Fig. 1). The areas were left undisturbed for 16 hours, and any sites of vasoconstriction were then noted. Three patterns of vasoconstriction were seen: (1) perifollicular (2) pallor at periphery of application site (3) diffusely in the circular area of application. The degree of vasoconstriction varied with test subject and compounds used, but no attempt was made to grade its intensity. Vasoconstriction was expressed as "present" or "absent." Figure 2 demonstrates the vasoconstriction produced by fluocinolone acetonide.

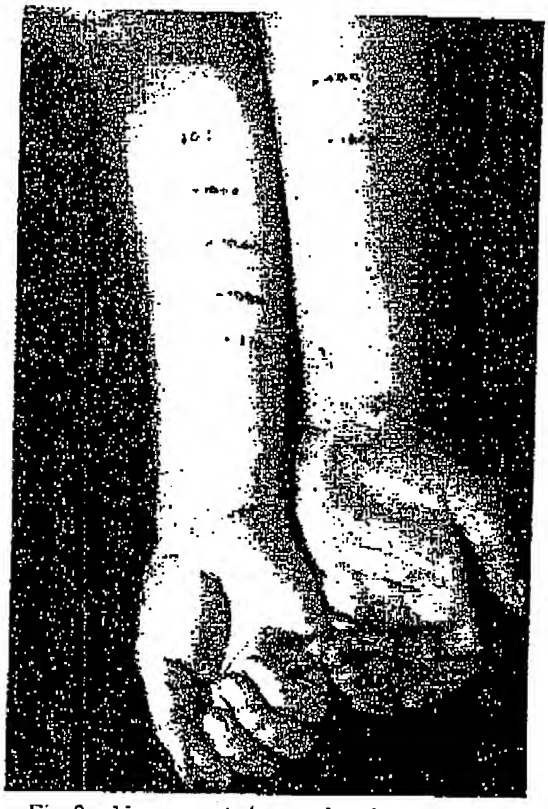

Fig. 2.—Vasoconstriction produced by dilutions of fluocinolone acetonide. Upper arm, Saran Wrap; lower arm, metal guard.

Results

Table 1 indicates the number of subjects reacting to the various dilutions under Saran

* Supplied by Merck Sharp & Dohme Research Laboratory, Pa.

† Supplied by E. R. Squibb & Sons, N.Y.

‡ Supplied by Syntex Laboratories Inc., N.Y.

TABLE 1.—*Subjects Showing Vasoconstriction at Serial Dilutions*

Steroid	No. of Subjects	Saran						Guard				
		1:100	1:1,000	1:10,000	1:100,000	1:1,000,000	1:4,000,000	1:100	1:1,000	1:10,000	1:100,000	1:1,000,000
Dexamethasone	13	13	13	11	5	0	0	9	1	0	0	0
Triamcinolone acetonide	11	11	11	11	10	7	0	11	11	8	0	0
Fluocinolone acetonide	8	8	8	8	8	8	2	8	8	7	0	0

Wrap, as compared with various dilutions under the perforated guard.

Table 2 shows the average "end-point" of reaction of these compounds under Saran Wrap and the metal guard. It can be seen that with occlusion this end-point differs by a factor of 100 over simple application of the compound.

TABLE 2.—*Average "End-Point" of Vasoconstriction*

Compound	Saran	Guard	Absorption Factor
Dexamethasone	1:10,000	1:100	× 100
Triamcinolone acetonide	1:1,000,000	1:10,000	× 100
Fluocinolone acetonide	1:1,000,000	1:10,000	× 100

Comment

These experiments appear to explain the efficiency of this method of topical therapy in terms of increased absorption. After occlusion of an arm with Saran Wrap for several hours there is obvious hydration of keratin; the hydration of keratin decreases and returns to normal in approximately 30 minutes when evaporation is allowed to proceed. Hydration of the skin has previously been shown to increase the percutaneous absorption of various substances (Rothman [6] and Cronin and Stoughton [7]). Temperature increase may also play a part since thermometers placed under the Saran Wrap for 16 hours showed that the occluded skin temperature equaled internal body temperature.

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